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Application of	:	HIROYUKI KOIKE et al
Serial No.	:	07/941,676
Filed	:	September 8, 1992
For	:	Tetrahydrothienopyridine derivatives, furo and pyrrolo analogs thereof and their preparation and uses for inhibiting blood platelet aggregation
Group	:	Art Unit 1203: P. Spivack

DECLARATION UNDER 37 CFR 1.132

I, Fumitoshi ASAI, of 3-5-17, Midori, Tanashi-shi, Tokyo, Japan, sincerely and solemnly declare as follows:

1. I graduated from the Department of Veterinary Science, the Faculty of Agriculture, the University of Tokyo, Japan, in March, 1977. I received a Ph.D. from the Faculty of Agriculture of the University of Tokyo in 1984. I entered into the employment of Sankyo Co., Ltd., Tokyo, Japan, in April, 1981 and am now an assistant chief researcher in Biological Research Laboratories of the said company. From December, 1984 through November, 1986, I studied at the Pharmacological Institute, the Faculty of Medicine, the University of Cologne, Cologne City, Germany.

I am a member of the following scientific societies:

The Japanese Pharmacological Society,

The Japanese Society of Veterinary Science,

The Japanese Society on Thrombosis and Hemostasis,

The Japanese Society of Hematology,

The Japanese Society for Circulation Research,

The Japan Society of Smooth Muscle Research.

Representatives of the scientific reports recently written by myself and my co-workers are as recited below.

(1) "RS-5186, a novel thromboxane synthetase inhibitor with a potent and extended duration of action."; Thromb. Res., 51, 507-520 (1988).

(2) "Role of activated platelets in endotoxin-induced DIC in rats."; Thromb. Res., 59, 735-747 (1990).

(3) "Reduced erythrocyte deformability in anemic rats with adjuvant arthritis."; Japan. J. Pharmacol., 530, 510-514 (1990).

(4) "Recombinant human erythropoietin, but not iron supplementation, improves anemia in rats with adjuvant-induced arthritis."; Japan. J. Pharmacol., 57, 291-298(1991).

2. I am one of the joint inventors for the above-identified application and thus have fully understood that the above-identified application has been rejected under 35 U.S.C. 103 over U.S. Patent No. 4,136,186, U.S. Patent No. 4,458,074 and

European Patent Publication No. 421861.

In order to show the difference between the subject matter of the cited references and the subject matter of the above-identified application, the following experiments were undertaken by myself and under my direction.

3. Experiments

(1) Inhibition of Blood Platelet Aggregation

The % inhibition of blood platelet aggregation of test compounds was determined according to the same method as the Test Example 2 in the specification of the above-identified application (at Pages 83-84). The results are shown in Table 1.

Table 1

Test Compound	% Inhibition		
	1 mg/kg	3 mg/kg	10 mg/kg
Compound C*)	-	0	0
Compound D**)	3.6	97.3	-
Compound E***)	3.8	97.9	-

*) Compound C (Compound in Example of U.S. Patent No. 4,458,074): 5-(2-chlorobenzyl)-2-oxo-2,4,5,6,7,7a-hexahydro-thieno[3,2-c]pyridine hydrochloride

**) Compound D (Compound in Example 19 of European Patent Publication No. 421861): (RS)-2-acetoxy-5-(2-chloro-α-

methoxycarbonylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride

***) Compound E (Compound in Example 4 of U.S. Patent No. 4,740,510): (RS)-5-(2-chloro-α-methoxycarbonybenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine

Our compounds in Examples 20 and 22 having the 2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine ring have much more potent blood platelet aggregation-inhibitory activity than Compound C in U.S. Patent No. 4,458,074 having the same ring. And our compounds in Examples 23, 25 and 26 having the 2-alkanoyloxy-4,5,6,7-tetrahydrothieno[3,2-c]pyridine ring have much more potent blood platelet aggregation-inhibitory activity than Compound D in European Patent Publication No. 421861 having the same ring. Also our compound in Examples 20 having a 2-fluoro-α-cyclopropylcarbonylbenzyl substituent at the 5-position of the 2-oxo-2,4,5,6,7,7a-hexahydrothieno-[3,2-c]pyridine ring have much more potent blood platelet aggregation-inhibitory activity than Compound E in U.S. Patent No. 4,740,510 having a 2-chloro-α-methoxycarbonylbenzyl substituent on the 5-position of the same ring.

(2) Preparation of 5-(2-chlorobenzyl)-2-oxo-2,4,5,6,7,7a-hexahydro-thieno[3,2-c]pyridine hydrochloride (Compound C)

(i) 5-Triphenylmethyl-4,5,6,7-tetrahydro-thieno[3,2-c]pyridine

3.5 g of triethylamine were added to a solution of 3.0 g of 4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride in 20 ml of methylene chloride, whilst stirring and then a solution of 4.8 g of triphenylmethyl chloride in 15 ml of methylene chloride was slowly dropwise added to the solution. The mixture was stirred at room temperature for 4 hours, washed with water, 0.1N hydrochloric acid, water and saturated aqueous sodium hydrogen carbonate and dried over anhydrous magnesium sulfate. The solvent was removed by evaporation under reduced pressure. The resulting residue was purified by column chromatography through silica gel using toluene as the eluent to afford 4.8 g of the title compound as white crystals, melting at 95°C.

Mass Spectrum (CI, m/z): 382 (M^++1), 243.

(ii) 2-Oxo-5-triphenylmethyl-2,4,5,6,7,7a-hexahydro-thieno[3,2-c]pyridine

10 ml of butyl lithium (1.6 M in hexane) were dropwise added to a solution of 5.4 g of triphenylmethyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridine in 40 ml of tetrahydrofuran, whilst cooling at 0°C over a period of 30 minutes and a solution of 4.3 ml of tributyl borate in 5 ml of tetrahydrofuran was dropwise added to the solution, whilst cooling in ice-sodium chloride bath over a period of 15 minutes. The mixture was stirred at room temperature for one hour and 3.3 ml of 30% aqueous hydrogen peroxide were added to the mixture, whilst cooling at -40°C over a period of 30 minutes. The mixture was allowed to stand at room temperature overnight. The reaction mixture was filtered by using celite.

The filtrate was washed with saturated aqueous sodium thiosulfate and saturated aqueous sodium chloride and dried over anhydrous magnesium sulfate. The solvent was removed by evaporation under reduced pressure. Diisopropyl ether was added to the residue, the mixture was stirred at room temperature and precipitated crystals were collected by filtration to afford 3.7 g of the title compound as pale brown crystals, melting at 210°C (with decomposition).

IR Spectrum, ν_{max} cm^{-1} (KBr): 1675.

NMR Spectrum, δ ppm ($CDCl_3$): 1.60-2.47 (4H, m), 3.27-3.39 (1H, m), 3.92-4.17 (2H, m), 6.09 (1H, s), 7.14-7.54 (15H, m).

Mass Spectrum (CI, m/z): 398 (M^++1), 243.

(iii) 2-Oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine hydrochloride

15 ml of formic acid were added to 3.1 g of 2-oxo-5-triphenylmethyl-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine and the solution was stirred at 90°C for one hour. Hydrogen chloride gas was bubbled into the solution, whilst ice-cooling and the solvent was removed by evaporation under reduced pressure. Diethyl ether was added to the resulting residue and stirred at room temperature, and precipitated crystals were collected by filtration to afford 1.5 g of the title compound as brown yellow crystals, melting at 210°C (with decomposition).

IR Spectrum, ν_{max} cm^{-1} (KBr): 1660.

NMR Spectrum, δ ppm ($CDCl_3$): 1.74-1.97 (1H, m), 2.55-2.68 (1H, m), 3.11-3.51 (2H, m), 3.97 (1H, d, J=15 Hz), 4.36 (1H, d, J=15 Hz), 4.71-4.83 (1H, m), 6.52 (1H, s), 9.95 (2H, br.s).

Mass Spectrum (CI, m/z): 156 (M^+-35).

(iv) 5-(2-Chlorobenzyl)-2-oxo-2,4,5,6,7,7a-hexahydro-thieno[3,2-c]pyridine and its hydrochloride

4.7 g of potassium hydrogen carbonate were added to a solution of 3.0 g of 2-oxo-2,4,5,6,7,7a-hexahydro-thieno[3,2-c]pyridine hydrochloride in 30 ml of dimethylformamide, 4.9 g of 2-chlorobenzyl bromide were added to the mixture and the mixture was stirred at room temperature for 2.5 hours. Ethyl acetate was added to the reaction mixture, and the mixture was washed with saturated aqueous sodium hydrogen carbonate for three times and dried over anhydrous magnesium sulfate. The solvent was removed by evaporation under reduced pressure The resulting residue was purified by column chromatography through silica gel using a 25:1 by volume mixture of toluene and ethyl acetate as the eluent to afford 2.8 g of the title compound as a pale brown oil.

NMR Spectrum, δ ppm ($CDCl_3$): 1.79-1.97 (1H, m), 2.34-2.56 (2H, m), 3.00-3.13 (2H, m), 3.72-3.90 (3H, m), 4.12-4.22 (1H, m), 6.05 (1H, s), 7.18-7.48 (4H, m).

Mass Spectrum (CI, m/z): 280 (M^++1).

The oil obtained above was dissolved in diethyl ether and treated with hydrogen chloride gas to afford the hydrochloride of the title compound as white crystals, melting at 99-104°C.

Elemental analysis:

Calculated for $C_{14}H_{14}ClNOS.HCl.1/2H_2O$:

C, 51.70%; H, 4.96%; N, 4.31%.

Found: C, 51.91%; H, 4.89%; N, 4.24%.

(3) Preparation of (RS)-2-acetoxy-5-(2-chloro-α-methoxycarbonylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine hydrochloride (Compound D)

(i) (RS)-5-(2-Chloro-α-methoxycarbonylbenzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine (Compound E)

Following a procedure similar to that described in the first step of Experiment (2)(iv), except that α-methoxycarbonyl-2-chlorobenzyl bromide was used in place of 2-chlorobenzyl bromide, the title compound was obtained as an orange oil in a yield of 36%.

NMR Spectrum, δ ppm ($CDCl_3$): 1.76-1.96 (1H, m), 2.30-2.42 (1H, m), 2.54-2.73 (1H, m), 2.96-3.33 (2H, m), 3.72 and 3.73 (total 3H, each s), 3.77-3.96 (1H, m), 4.11-4.20 (1H, m), 4.89 and 4.91 (total 1H, each s), 6.00 and 6.02 (total 1H, each s), 7.12-7.56 (4H, m).

Mass Spectrum (CI, m/z): 338 (M^++1).

Diisopropyl ether was added to the oil obatined above and stirred at room temperature to afford white crystals, melting at 111 - 113.5°C.

Elemental analysis:

Calculated for $C_{16}H_{16}ClNO_3S$:

C, 56.88%; H, 4.77%; N, 4.15%.

Found: C, 56.86%; H, 4.67%; N, 4.19%.

(ii) (RS)-2-Acetoxy-5-(2-chloro-α-methoxycarbonylbenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine and its hydrochloride

0.19 g of sodium hydride (60% in mineral oil) was added to a solution of 1.5 g of 5-(2-chloro-α-methoxycarbonyl-benzyl)-2-oxo-2,4,5,6,7,7a-hexahydrothieno[3,2-c]pyridine in 9 ml of dimethylformamide and in 3 ml of acetic anhydride, whilst ice-cooling and the mixture was stirred at same temperature for 20 minutes and at room temperature for 3 hours. Ethyl acetate was added to the reaction mixture, washed with saturated aqueous sodium hydrogen carbonate and saturated aqueous sodium chloride and then dried over anhydrous magnesium sulfate. The solvent was removed by evaporation under reduced pressure. The resulting residue was purified by column chromatography through silica gel using a 100:3 by volume mixture of toluene and ethyl acetate as the eluent to afford 1.2 g of the title compound as a colorless oil. Diisopropyl ether was added to the oil obtained above and stirred at room temperature to afford white crystals, melting at 85-86.5°C.

NMR Spectrum, δ ppm ($CDCl_3$): 2.26 (3H, s), 2.73-2.84 (2H, m), 2.85-2.94 (2H, m), 3.49-3.72 (2H, m), 3.73 (3H, s), 4.92 (1H, s), 6.27 (1H, s), 7.25-7.75 (4H, m).

Mass Spectrum (CI, m/z): 380 (M^++1).

Elemental analysis:

Calculated for $C_{18}H_{18}ClNO_4S$:

C, 56.91%; H, 4.78%; N, 3.69%.

Found: C, 56.92%; H, 4.60%; N, 3.70%.

Following a procedure similar to that described in the second step of Experiment (2)(iv), the hydrochloride of the title compound was obtained as white crystals, melting at

138-142°C.

Elemental analysis:

Calculated for $C_{18}H_{18}ClNO_4S.HCl$:

C, 51.93%; H, 4.60%; N, 3.37%.

Found: C, 52.03%; H, 4.50%; N, 3.35%.

4. Conclusion

These data clearly show that our corresponding Compounds have much more potent blood platelet aggregation-inhibitory activity than Compound C in U.S. Patent No. 4,458,074 and Compound D in European Patent Publication No. 421861.

5. I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willfull false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statemnts may jeopardize the validity of the application or any patent issued thereon.

Fumitoshi Asai

Fumitoshi Asai, Ph.D.

Date : April 20, 1993